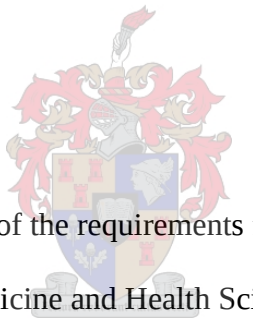
The value of repeat lumbar cerebrospinal fluid analysis in the diagnosis of childhood TBM

Madelein Grobbelaar



Thesis presented in partial fulfillment of the requirements for the Degree of Master of Medicine (Paediatrics) in the Faculty of Medicine and Health Sciences, at Stellenbosch University

Supervisor: Prof R Solomons

Co-supervisor: Prof R van Toorn

December 2019

Stellenbosch University https://scholar.sun.ac.za

Declaration

I, the undersigned, hereby declare that the work contained in this assignment is my original work and that I have not previously submitted it, in its entirety or in part, at any university for a degree.

Signature: .....

Date: .....

Copyright © 2019 Stellenbosch University

All rights reserved

Stellenbosch University https://scholar.sun.ac.za

Abstract

Objective: Early diagnosis and treatment initiation in childhood tuberculous meningitis (TBM) remains sub-optimal and contributes significantly to morbidity and mortality in this age group, especially in TB endemic areas. We aimed to describe the evolution of lumbar CSF parameters analyzed serially, to determine if re-sampling would influence the diagnostic accuracy of childhood TBM.

Methods: We performed a retrospective observational study which included children with suspected TBM that were prospectively enrolled in several TBM research studies, at Tygerberg Hospital, Cape Town, South Africa, over a 30-year period. A data collection sheet was used to compare demographic, clinical and diagnostic characteristics on admission and subsequent repeat CSF parameters at weeks 1, 2 and 3.

Results: Of 318 children with suspected TBM, 53(17%) patients had 'definite' TBM and 265(83%) patients had 'probable' TBM. Serial CSF analysis demonstrated a decrease in mean lymphocyte (143 to 49/µL) and neutrophil count (53 to 9/µL) over the 3-week period. Mean CSF protein also showed a gradual decline (3.29 to 1.85g/L) with a rise in mean CSF glucose (1.89 to 2.72 mmol/L) three weeks after initiating treatment. A total of 230 (72%) patients were identified that had atypical CSF trends; an increase in CSF lymphocyte, neutrophil and protein count after TBM treatment initiation. A history of prolonged illness >5days ($p=0.01$) and initial CSF neutrophil predominance ($p<0.01$) were associated with an atypical increase in serial CSF lymphocyte count, while a positive tuberculin skin test ($p=0.05$) and initial CSF lymphocyte predominance ($p<0.01$) were associated with an atypical increase in serial neutrophil count. Admission CSF protein level >1g/L ($p<0.01$) predicted an atypical serial increase in CSF protein. According to the uniform research case definition for TBM, 14% of patients with "possible" TBM, would have improved their diagnostic status to "probable" TBM after repeat CSF analysis.

Conclusion: Repeat lumbar CSF in TBM suspects can demonstrate different trends over time. The typical trend is a gradual decline in CSF lymphocyte, neutrophil, and protein count whilst the CSF glucose rise steadily. It is not abnormal for the CSF lymphocyte, neutrophil, and protein

Stellenbosch University https://scholar.sun.ac.za

count to rise initially after treatment initiation, which can be seen as atypical for TBM. Although serial lumbar CSF analysis improves the diagnosis of childhood TBM when the uniform TBM research case definition's scoring system is applied, it does not improve early diagnosis and outcome and could cause confusion if an atypical trend is found.

Key words: Tuberculous meningitis, Cerebrospinal fluid, diagnosis

Stellenbosch University https://scholar.sun.ac.za

Opsomming

Mikpunte: Vroeë diagnose en behandeling van tuberkuleuse breinvliesontsteking in kinders bly sub optimaal en dra betekenisvol by tot morbiditeit en mortaliteit, veral in hoë TB-belaste nasies. Ons het beoog om die verandering in serebrospinale vog (SSV) parameters oor ‘n periode te beskryf om sodoende te bepaal of herhaal lumbale punksie ‘n merkwaardige invloed sal hê in die diagnose van kinder tuberkuleuse breinvliesontsteking.

Metodes: Retrospektiewe ontleding van 318 kinders met ‘n suspisie van tuberkuleuse breinvliesontsteking oor ‘n 30 jaar periode, by die Tygerbergse Hospitaal, Kaapstad, Suid-Afrika. Demografiese, kliniese en diagnostiese eienskappe met opname, asook herhaal SVV ontleding 1, 2 en 3 weke later, is met mekaar vergelyk.

Uitslae: Uit die 318 kinders met ‘n suspisie van tuberkuleuse breinvliesontsteking is 53(17%) gevalle bakteriologies bevestig en 265(83%) geïdentifiseer as waarskynlik tuberkuleuse breinvliesontsteking. SSV ontleding oor die 3-week periode het ‘n toename in gemiddelde limfosiet (143 na 49/µL) en neutrofiel telling (53 na 9/µL) getoon. Die gemiddelde proteïen konsentrasies het ook gelydelik afgeneem (3.29 to 1.85g/L) terwyl die SSV glukose vermeerder het oor die periode (1.89 na 2.72 mmol/L). Atipiese SSV patrone is gevind in 230 (72%) pasiënte na aanvang van behandeling, dit sluit in ‘n toename in SSV limfosiet, neutrofiel en proteïen telling. ‘n Geskiedenis van verlenge siekte >5 dae ($p=0.01$) en oorheersende SSV neutrofiele ($p<0.01$) was geassosieer met atipiese toename in SSV limfosiet telling, terwyl ‘n positiewe tuberkulien vel toets ($p=0.05$) en oorheersende SSV limfosiet ($p<0.01$) telling geassosieer was met atipiese toename in SSV neutrofiel telling. Aanvanklike proteïen konsentrasie >1g/L ($p<0.01$) was geassosieer met ‘n atipiese toename in SSV proteïen. Volgens die univorme navorsings gevalsdefinisie van tuberkuleuse meningitis, sou 14% van pasiënte met “moontlike” tuberkuleuse breinvliesontsteking, met herhaling van SVV, verander het na “waarskynlik” tuberkuleuse breinvliesontsteking.

Stellenbosch University https://scholar.sun.ac.za

Gevolgtrekking: Herhaal SSV in kinders met die suspisie van tuberkuleuse breinvliesontsteking kan verskillende patrone wys. Die klassieke SSV patroon toon ‘n afname in limfosiet, neutrofiel en proteïen konsentrasies, met ‘n toename in SSV glucose. Aanvanklike toename in SSV limfosiet, neutrofiel en proteïen konsentrasies, na aanvang van behandeling, is wel bekend. Hierdie beeld kan as atipiese gesien word vir tuberkuleuse breinvliesontsteking. Alhoewel herhaal SSV die diagnose van tuberkuleuse breinvliesontsteking verbeter wanneer die univorme navorsings gevalsdefiniëring van tuberkuleuse meningitis gebruik word, dra dit nie by tot vroeër diagnose en beter uitkomste in kinders met tuberkuleuse breinvliesontsteking, en kan ‘n atipiese SSV patroon diagnostiese verwarring veroorsaak.

Stellenbosch University https://scholar.sun.ac.za

Acknowledgement

I would like to thank Professors Regan Solomons and Ronald van Toorn for their support, guidance, and insight throughout this research project.

A special word of thanks to Professor Robert Gie, whose experience, knowledge and enthusiasm is inspiring.

To the Department of Paediatrics at Tygerberg Hospital, thank you for the opportunity as post-graduate student of the University of Stellenbosch.

Stellenbosch University https://scholar.sun.ac.za

Dedications

‘We all need people in our lives who raise our standards, remind us of our essential purpose, and challenge us to become the best version of ourselves’ Matthew Kelly

Thank you to Johann, my family, the van der Merwe family, and my friends.

Stellenbosch University https://scholar.sun.ac.za

Table of Contents

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

List of Abbreviations

TBM:	Tuberculous meningitis
CSF:	Cerebrospinal fluid
WHO:	World Health Organization
TB:	Tuberculosis
PTB:	Pulmonary tuberculosis
HIV:	Human immunodeficiency virus
CNS:	Central nervous system
GCS:	Glasgow coma scale
DNA:	Deoxyribonucleic acid
CT:	Computed tomography
MRI:	Magnetic resonance imaging
HREC:	Health Research Ethics Committee
BMRC:	British Medical Research Council

Stellenbosch University https://scholar.sun.ac.za

Chapter 1: Introduction

Tuberculous meningitis (TBM) is one of the most severe complications of tuberculosis and frequently occurs in children. Diagnostic uncertainty and treatment initiation remains a problem in childhood TBM resulting in substantial neurological morbidity or death. TBM is classically described as a subacute lymphocytic meningitis but the variety of clinical presentation, non-specific symptoms, and young age at presentation, together with the limitation and challenges of diagnostic techniques available, all contribute in clouding the diagnosis. The development of a uniform research case definition has significantly contributed to guide clinicians and researchers to standardize a diagnosis for TBM.

Diagnosis can neither be made or excluded on clinical grounds and is dependent on cerebrospinal fluid (CSF) analysis. Typical CSF findings in TBM suspects have been well-described but atypical CSF findings are commonly found. The diagnosis is regularly based only on clinical and preliminary cerebrospinal fluid (CSF) findings without any definitive microbiologic confirmation of *Mycobacterium tuberculosis.*

Minimal data exist on the evolution of CSF changes, as determined by serial analysis, in childhood TBM. Knowledge of serial changes in the CSF parameters of children with TBM could possibly increase both the sensitivity and specificity in the diagnosis of TBM in children.

By analyzing the changes in all lumbar CSF parameters on admission, week 1, week 2 and week 3, one can possibly determine normal CSF evolution in patients with “probable” and “definite” TBM. This can assist and guide healthcare workers with a reference point, aiding in the diagnosis of childhood TBM. By assessing the atypical CSF trends, one can possibly identify which clinical features could be predictors.

Stellenbosch University https://scholar.sun.ac.za

Chapter 2: Literature review

According to the World Health Organization (WHO) 2015 Global Tuberculosis (TB) Report, mortality in TB has fallen 47% since 1990 due to improved diagnosis and treatment. The TB incidence has also declined and is currently 18% lower than in 2000.[1] An estimated 9.6 million people developed TB in 2014, of whom approximately one tenth are children. In South Africa, extrapulmonary TB accounts for 10 % of TB cases and children under 15 years contribute 10% of the total TB caseload.[1]

Despite recent advances, TB remains a global threat. Early diagnosis and treatment initiation in children with tuberculous meningitis (TBM) remains sub-optimal, contributing significantly to an increased mortality and morbidity.

Tuberculous meningitis represents 1% of all cases of TB. This number is disproportionately small, compared to the resultant morbidity and mortality.[2] Young children and patients with immunodeficiency, mostly HIV co-infection and cancer, are at increased risk of developing TBM. Other factors, amongst others, that contributed to the development of TB are malnutrition, anaemia, advanced immune suppression and frequent TB exposure.[3-5] The peak age for TBM incidence is <5 years.[6-8]A large South African retrospective study of children diagnosed with TBM found that 82% of children were < 5 years of age.[7] In the Western Cape Province of South Africa, TBM is the most common and most devastating form of paediatric bacterial meningitis.[9]

TBM is caused by *Mycobacterium tuberculosis (M.tb),* an organism with a lipid-rich cell wall. Rich and McCordock proposed the 2-step model for the pathogenesis of CNS TB. According to their model, based on autopsy findings, the majority of TBM cases had a single caseous focus (Rich's focus) that could be found from which, when ruptured, bacilli could spread to the subarachnoid space.[10] This has influenced the understanding of the pathogenesis of TBM, however, does not fully explain the frequency of miliary TB and TBM simultaneously occurring.[11]

Stellenbosch University https://scholar.sun.ac.za

TBM is typically a subacute disease, mostly with an insidious onset causing non-specific symptoms, delaying the early recognition and diagnosis. A prominent clinical feature of TBM is the sub-acute duration of symptoms, typically >6 days.[7, 12-14] TBM may also present acutely, or conversely have a chronic presentation with symptom duration ranging from 3 days to 6 months.[15] For clinicians to make an early diagnosis of TBM they should have a high index of diagnostic suspicion when faced with a child with non-specific symptoms and signs of possible neurological involvement.

In the prodromal phase of TBM, one or more of low-grade fever, malaise, headache, dizziness, vomiting, poor feeding and growth faltering, cough, irritability, and/or personality changes can persist for several weeks. Following the non-specific prodromal stage, children with TBM develop more central nervous system (CNS) specific symptoms including neck stiffness, depressed level of consciousness, raised intracranial pressure, motor deficit and cranial neuropathies. When TBM is allowed to progress without treatment, coma will ensue. Seizures, in contrast to adults, are commonly seen in children with TBM, occurring in up to half of pediatric cases. [7, 12,14,15] A history of recent close contact with an adult source-case with TB is usually found in half of children (30%-53%), more frequently than when compared to adult patients.[7,14,15]

Staging of the severity of TBM is based on the refined British Medical Research Council (BMRC) criteria. The staging criteria are as follows:

Stage I) Glasgow coma scale (GCS) of 15, without focal neurological deficit,
Stage IIa) GCS of 15 with focal neurological deficit
Stage IIb) GCS of 14-11 with or without focal neurological deficit
Stage III) GCS < 11 with or without focal neurological deficit.[16,17]

The signs and symptoms of stage I TBM disease are non-specific and similar to those of the primary infection rather than of neurological disease, commonly leading to missed diagnosis.[18] The majority of patients present with stage II or III TBM, with very poor outcome.[7,15]

Few patients (3-26%) present with early stage disease and unfortunately, the presenting clinical

Stellenbosch University https://scholar.sun.ac.za

symptoms sometimes complicates it further by not distinguishing TBM from other forms of meningitis, especially partially-treated pyogenic meningitis, resulting in diagnostic and treatment delay.[13, 19]

Despite many recent advances, diagnostic uncertainty remains a problem in childhood TBM. The diagnosis is regularly based only on clinical and preliminary cerebrospinal fluid (CSF) findings without definitive microbiologic confirmation of *M.tb* being available. Clinicians are often reluctant to start a patient, especially a child, on months of treatment without firm evidence of disease.

The non-specific clinical features of the disease, the low sensitivity of conventional microbiology due to the paucibacillary nature of CSF[20], and the lack of viable alternative diagnostic methods, all contribute to the diagnostic difficulty. A uniform case definition for TBM has enabled standardized TBM diagnosis for research purposes, especially in view of the limitation and challenges of diagnostic techniques available. Patients are stratified as having definite, probable or possible TBM using their clinical presentation together with the laboratory and radiological findings.[21]

Lumbar CSF analysis is essential to the diagnosis of TBM. Characteristic lumbar CSF findings of TBM include: 1) clear and colorless appearance 2) increased leucocyte count of 10-500 cells/µL 3) lymphocyte predominance of >50% 4) raised protein (>1.0 g/L) and 5) decreased glucose concentration (absolute value < 2.2 mmol/L and/or CSF-plasma ratio <50%).[7,13,19,21-23] Atypical CSF findings are common, including normal CSF cell count, protein and/or glucose levels, and even a neutrophil predominance.[13] Differentiating tuberculous meningitis from other forms of bacterial meningitis in TB-endemic environments can be challenging.[13,19] For this reason clinicians often repeat a lumbar puncture to obtain CSF for analysis, especially if there is diagnostic uncertainty.

The CSF findings are further complicated due to the inconsistency between lumbar and ventricular CSF in patients with non-communicating hydrocephalus. Ventricular CSF may record normal chemistry and cells if drawn from a site proximal to inflammation and obstruction.

Stellenbosch University https://scholar.sun.ac.za

TBM is a chronic inflammatory response and CSF therefore takes weeks to normalize whilst rapid normalization is expected in non- tuberculous meningitis. CSF changes following initiation of anti-tuberculous treatment in children are not well-described. Previous adult studies that have examined serial CSF changes in patients with TBM found that: 1) the CSF normalizes over time, but the rate is variable ranging from 1-32 months 2) CSF glucose normalizes rapidly 3) CSF lymphocyte count and protein concentration changes slowly 4) CSF neutrophil count rapidly normalizes 5) CSF can temporarily worsen after anti-tuberculous treatment is initiated, i.e. CSF leucocyte count rises during the first 7 days, and 6) there is no correlation between the rate of change in CSF and TBM stage or the clinical response of the patient.[25-27] These changes in the CSF profile provide an opportunity to increase the diagnostic accuracy of TBM. Repeat CSF analysis may provide valuable clinical guidance when deciding between TBM or an alternate cause of meningitis. If both CSF neutrophil count and glucose do not serially improve, it should be considered atypical for TBM and an alternative diagnosis, or drug-resistant strain of *M.tb* should be considered.[28]

Corticosteroids not only improve clinical outcome and mortality rate in tuberculous meningitis but significantly lower CSF protein and globulin levels after 1 month of treatment and cause a steadier rise in CSF glucose levels compared to a non-steroid therapy group.[29] Serial CSF cell counts, however, are not affected by adjuvant steroid therapy.[29]

HIV modifies CSF characteristics in tuberculous meningitis. HIV-infected patients with TBM has a higher frequency of CSF characteristics that indicate a non-inflammatory CSF (absence of pleocytosis) and lower CSF protein levels, but similar CSF glucose levels when compared to non-infected patients.[20] With HIV co-infection, there is a higher frequency of multidrug-resistant strains (MDR) which contributes significantly to the increase in mortality rate (63.3% vs 17.5%).[30]

Mycobacterial DNA may be detectable in the CSF for up to a month following initiation of treatment, while the sensitivity of CSF smear and culture decreases rapidly.[31] The diagnosis of “definite” TBM, remains mycobacteriological confirmation in the CSF by microscopic identification of acid-fast bacilli, culture of *M.tb* and/or a positive commercial nucleic-acid

Stellenbosch University https://scholar.sun.ac.za

amplification test for *M.tb.*[21] It is well-recognized that the sensitivity of acid-fast bacilli staining and positive mycobacterial CSF culture depends on the number and volume of CSF samples obtained for analysis. A single sample has low sensitivity, 20%–40%.[32] Two studies found that the sensitivity of positive mycobacterial CSF culture improved from 53% to 83%, after examining multiple samples of CSF.[33-34]

In 2013, the WHO recommended that Xpert MTB/RIF® be used for rapid diagnosis in preference to conventional microscopy and culture as the initial diagnostic test in all TBM suspects.[35] Caution, however, is advised especially due to low sensitivities, and it is recommended that larger CSF volumes are sampled.[36]A study by Solomons et al. found that more than one commercial nucleic-acid amplification test incrementally increased CSF diagnostic accuracy in childhood TBM.[37] It would not be illogical to perform commercial nucleic-acid amplification tests on serial CSF samples in order to improve overall sensitivity and specificity.

The outcome in TBM can only be improved through early diagnosis and treatment initiation, which can be aided by developing novel diagnostic tests and refining existing diagnostic methods. Research in childhood TBM is lacking on the evolution of CSF changes over time, as determined by serial CSF analysis.

Stellenbosch University https://scholar.sun.ac.za

Chapter 3: Aim of the investigation

3.1 Research justification:

Childhood TBM remains a devastating disease and a global threat, especially in a TB endemic area like the Western Cape Province of South Africa. The majority of children diagnosed with TBM are <5 years of age. Demographic, clinical, and social factors have been identified which contributes to the increased risk of developing childhood TBM.

Despite many advances, diagnostic uncertainty remains a problem in childhood TBM. The non-specific clinical features of the disease, the low sensitivity of conventional microbiology due to the small numbers of bacilli in the CSF, and the lack of viable alternative diagnostic methods, all contribute to the diagnostic difficulty. This results in delay of treatment initiation and disease progression. The majority of patients unfortunately present with advanced stage disease, with very poor outcome.

The outcome in TBM can be improved through early diagnosis and treatment initiation, which can be aided by developing novel diagnostic tests and refining existing diagnostic methods.

Previous adult studies that have examined serial CSF changes following initiation of anti-tuberculous treatment have shown that repeat CSF analysis can provide valuable clinical guidance with regards to diagnostic accuracy. The sensitivity for acid-fast bacilli staining and positive mycobacterial CSF culture can also be improved, after examining multiple samples of CSF.

Research in childhood TBM is lacking on the evolution of CSF changes, as determined by serial analysis in children being treated for suspected TBM.

Stellenbosch University https://scholar.sun.ac.za

3.2 Research hypotheses:

Repeat CSF analysis in children with possible or probable TBM, does not improve diagnostic accuracy.

3.3 Research question:

Does serial lumbar CSF re-sampling influence the diagnostic accuracy of childhood TBM?

3.4 Primary research outcomes:

1. To describe the evolution of CSF parameters analyzed serially, that affect the sensitivity of clinical confirmation in children diagnosed with TBM.

3.5 Secondary outcomes:

1. To describe the demographic profile of the study population i.e. age, race, and gender
2. To describe changes in all CSF parameters on admission, week 1, week 2 and week 3
3. To identify the optimal timing for CSF re-sampling in TBM suspects with atypical initial CSF analysis
4. To determine which clinical factors are associated with a serial increase in CSF leucocytes and/or protein, and/or serial decrease in CSF glucose in childhood TBM suspects

Stellenbosch University https://scholar.sun.ac.za

Chapter 4: Methodology

4.1 Setting:

The study was conducted at Tygerberg Hospital, Cape Town. It is currently the largest hospital in the Western Cape, and the second largest hospital in South Africa serving as the academic and teaching hospital for Stellenbosch University. It lies within the metropole of Cape Town serving 2.6 million people, mostly poor communities. Each year, approximately 16 000 neonates and children receive inpatient medical care at Tygerberg Hospital and more then 100 000 receive specialist medical care as outpatients.

4.2 Study population:

Children prospectively enrolled in previous TBM research studies at Tygerberg Hospital between January 1985 and December 2015 were included.

4.2.1 Inclusion criteria

1) All children aged between 3 months and 13 years
2) Any clinical suspicion of tuberculous meningitis (TBM)
3) Children with CSF analysis on admission
4) Children with at least one further CSF analysis at either 1, 2 or 3 weeks post-admission
5) Children with drug susceptible TBM

Stellenbosch University https://scholar.sun.ac.za

4.2.2 Exclusion criteria

1) Children aged less than three months and older than 13 years
2) Non-tuberculous meningitis
3) Children with confirmed or suspected drug resistant TBM

4.3 Comparator

Serial lumbar puncture CSF analysis

4.4 Outcome

1) A change in TBM CSF over time
2) To determine which CSF values over time are suggestive of an alternate diagnosis.
3) To determine which clinical factors are associated with an atypical TBM trend

4.5 Time frame

A 30-year period between 01 January 1985 and 31 December 2015

4.6 Data collection

The primary sources of data were obtained from an existing TBM database with previously consented studies (HREC reference numbers N10/11/367 and N11/01/006).

Stellenbosch University https://scholar.sun.ac.za

This included:

4.6.1. Demographic data: age, gender, race

4.6.2. Medical data: BCG, TB contact, HIV status, nutrition

4.6.3. Clinical data: TBM Stage of disease, duration of symptoms, fever, weight loss, vomiting, convulsions, cough, headache, failure to thrive, night sweats, altered level of consciousness, GCS on admission, meningeal irritation, raised intracranial pressure, brainstem dysfunction, cranial nerve palsies, and focal neurological deficit

4.6.4. Special investigations: Tuberculin skin test, CXR, gastric washings, neuroimaging, CSF analysis. The specific CSF characteristics that were collected included CSF cell count, CSF glucose concentration, CSF plasma glucose ratio, CSF protein concentration, CSF microbiology results including CSF acid fast bacilli microscopy, CSF MTB culture, CSF molecular diagnostic result.

4.7 Case-definitions used

4.7.1 Diagnosis of TBM

Patients was classified as probable or definite TBM according to the uniform research case definition based on a scoring system consisting of clinical, CSF, and neuroimaging findings (Appendix 2).[21]

A maximum score of 4 points is assigned to CSF criteria which includes

1) clear CSF appearance
2) leucocyte count 10-500 cells/µL
3) lymphocytic predominance (>50%)
4) protein concentration >1g/L
5) CSF: plasma glucose ratio < 50% or an absolute CSF glucose value< 2.2mmol/L

Stellenbosch University https://scholar.sun.ac.za

Possible TBM

TBM was classified as 'possible' when a patient had a diagnostic score of 6–11 when neuroimaging was available and 6–9 when neuroimaging was unavailable.[21]

Probable TBM

TBM was classified as 'probable' when patients scored ≥ 12 when neuroimaging was available and ≥10 when neuroimaging was unavailable.[21]

Definite TBM

TBM was classified as 'definite' when CSF demonstrated acid-fast bacilli and/or positive *M. tuberculosis* culture and/or positive commercial nucleic acid amplification test for *M. tuberculosis* in a patient with symptoms or signs suggestive of the disease.[21]

4.7.2 TBM Stage: TBM was staged according to the refined British Medical Research Council (BMRC) criteria as:

- Stage I: Glasgow Coma Scale (GCS) of 15 and no focal neurology
- Stage IIa: GCS of 15 plus focal neurology
- Stage IIb: GCS of 11-14 with focal neurology
- Stage III: GCS <11[17,18]

4.7.3 Atypical TBM CSF trends

1) CSF lymphocyte count increasing over time
2) CSF neutrophil count increasing over time
3) CSF protein count increasing over time
4) CSF glucose concentration decreasing over time

Stellenbosch University https://scholar.sun.ac.za

4.8 Data Management

Only retrospective routinely-collected data from an existing TBM database with previously consented studies by the Human Research Ethics Committee of Stellenbosch University (HREC reference numbers N10/11/367 and N11/01/006) were analyzed in this study. Data was captured on a data collection sheet (Appendix 1). To ensure complete confidentiality and anonymity, every patient in the existing TBM database was allocated a unique study number. The data was then transferred to an Excel spreadsheet for analysis. All information/data had a separate password protected file and this was only known to and accessed by the researchers

4.9 Data Analysis

Data analysis was done in collaboration with the Biostatistics Department at the University of Stellenbosch. Statistical analysis was performed using Statistical Package for the Social Sciences version 24 (SPSS Inc, Chicago, IL, USA). For numerical variables, mean and standard deviation were used for data with a normally distribution; median and interquartile range were used for data with a skewed distribution. Categorical variables were described using proportions. The X^2 and one-way ANOVA was used to assess differences between continuous and categorical variables respectively. The level of significance was set at the 5% level ($p<0.05$). Longitudinal clustering was done using the median.

To test the validity of the analysis, the cases with “possible” TBM, initially excluded from the database, were later compared to the results obtained from the “probable” and “definite” TBM cases. The repeat CSF results of the “possible” group were used in the recalculation of the case definition scoring system.

Stellenbosch University https://scholar.sun.ac.za

4.10 Ethical considerations

The study was approved by the Human Research Ethics Committee of Stellenbosch University, South Africa. Study reference no. S16/08/159. (Appendix 3)

Stellenbosch University https://scholar.sun.ac.za

Chapter 5: Results

In total, 318 children suspected of having TBM were included in this study, fifty-three (17%) patients had 'definite' TBM and 265 (83%) patients had 'probable' TBM.

Demographic data are shown in Table 1. The mean age at presentation was 36 months (range 2-180 months), males being slightly more affected than females (52% vs 48 %). There was a higher incidence amongst patients of mixed ancestry (251 patients; 79%). The HIV status was only available in 111 out of 318 patients (35%). Of these, 4 (4%) patients were HIV-infected. A background history of contact with a known household TB source case was found in 219 (69%) of patients.

Clinical features present on admission are summarized in Table 2. Prolonged symptom duration >5 days (76%), history of fever (63%) and altered level of consciousness (95%) were the most frequent presenting symptoms. Weight loss and convulsions was seen in less than half of the patients.

The most notable signs on admission were meningeal irritation (96%), abnormal GCS (96%) and motor deficit (63%). The majority of patients were classified as BMRC stage IIb (47%) and stage III (49%).

The tuberculin skin test was positive in 38% and abnormal chest X-ray findings were present in 61% of patients. Of these, 49% had findings suggestive of PTB, including intrathoracic lymphadenopathy and parenchymal consolidation, whilst the remaining 12% had miliary infiltration. Culture for *M.tb* from gastric washings were positive in 19%. Common neuroimaging features of TBM included hydrocephalus (92%) and basal meningo-vascular enhancement (78%).

Stellenbosch University https://scholar.sun.ac.za

CSF analysis was performed on admission and repeated at least once at either 1, 2 or 3 weeks post-admission. A total of 972 lumbar CSF samples were analyzed. All patients (318) had CSF analysis on admission, 260 samples (82%) were taken after 1 week, 198 (62%) after 2 weeks, and 196 samples (62%) were collected and analyzed 3 weeks after initiating treatment. Only 91 patients (29%) had complete CSF analysis done at all four time points. Table 4 summarizes all the CSF results on admission and changes in CSF cellular and biochemical parameters over time are shown in Figure 1.

On admission, a CSF cell count of 10-500/ µL was found in 276 patients (87%) and lymphocytic predominance in 261 (82%). Raised protein concentration of ≥ 1 g/L was found in 299 patients (72%). There were too few data available on serum glucose levels (n=56), but 222 (73%) had a CSF glucose ≤ 2.2 mmol/L, or CSF glucose/serum glucose ≤ 50%, when available. After one week on treatment, the lymphocyte, neutrophil, and protein count had decreased, and the CSF glucose concentration increased.

CSF analysis 3 weeks after treatment demonstrated a decline in both lymphocyte and neutrophil counts from 143/µL (95% CI, 116.22 -169.82) to 49/ µL (95% CI, 36.53-61.73) and 53/ µL (95% CI, 29.39-77.42) to 9/ µL (95% CI, 2.45-16.04) respectively (figure 1a and 1b). The mean CSF protein level showed a gradual decrease from 3.29g/L (95% CI, 1.62-4.96) on admission to 1.85g/L (95% CI, 1.48-2.26) three weeks after initiating treatment (figure1c). The mean CSF glucose gradually increased from 1.89 mmol/L (95% CI, 1.65-2.12) on admission to 2.72 mmol/L (95% CI, 2.59-2.86) after the 3rd week of treatment (figure 1d). Microbiological confirmation of *M.tb* in all CSF samples, either through microscopy, culture or PCR, was 17%.

Closer analysis of all data revealed two CSF trends. Eighty-eight (28%) of patients with "probable" or "definite" TBM had a typical, expected CSF trend of all cell lines and biochemical parameters over the three-week period. Typical CSF trends over time are shown in Figure 2.
A decrease in lymphocyte count was seen in 206 patients (65%), a decrease in neutrophil count was seen in 194 (61%) of patients, a decrease in protein count in 164 (52%) and an increase in CSF glucose concentration was found in 281 (93%) of patients.

Stellenbosch University https://scholar.sun.ac.za

On admission, a CSF lymphocytic predominance of 167/ µL (95% CI, 145.65-188.29) was found in 206 patients (65%), this decreased with more than half after the first week to 77/µL (95% CI, 63.08-91.90) and further decreased over the next two weeks to a count of 39 / µL (95% CI, 45.60-64.80) at the end of the 3 weeks (figure 2a). A mean CSF neutrophil count also demonstrated a decline 3 weeks after treatment from 66 / µL (95% CI, 50.58-80.98) to 4 / µL (95% CI, 1.75-5.94), with more than three quarter decline after 1 week (figure 2b). The mean CSF protein level showed a gradual decrease from 4.14g/L (95% CI, 2.85-5.43) on admission to 1.35g/L (95% CI, 1.05-1.64) three weeks after initiating treatment (figure 2c). The mean CSF glucose gradually increased form 1.68 mmol/L (95% CI, 1.56-1.79) on admission to 2.71 mmol/L (95% CI, 2.60-2.82) after the 3rd week of treatment (figure 2d).

A total of 230 (72%) patients were identified that had atypical CSF trends, of these 112 (49%) patients had an atypical lymphocyte trend, 123 (53%) an atypical neutrophil trend, 152 (66%) an atypical protein trend and 22 (10%) patients an atypical glucose trend. Figure 3 demonstrates atypical CSF changes in cellular and biochemical parameters over time.

On admission, a mean lymphocytic count of 86 / µL (95% CI, 68.41-103.39) increased to 150 µL (95% CI, 126.81-175.00) after one week, followed by a decreased over the next 2 weeks to 74/ µL (95% CI, 55.70-91.51) (figure 3a). An initial mean neutrophil count of 12/ µL (95% CI, 7.03-17.44), increased to 38/ µL (95% CI, 26.25-49.07) after 1 week, then decreased to 13 µL (95% CI, 8.32-17.36) and remained at a mean of 13/ul (95% CI, 5.50-20.84) after the 3 weeks of treatment (figure 3b). A mean protein concentration of 1.45 g/L (95% CI, 1.25-1.66) on admission increased over the first 2 weeks to a mean of 2.81g/L (95% CI, 1.81-3.82) and remained high at 2.11g/L (95% CI, 1.59-2.62) at the end of the treatment period (figure 3c). A mean glucose of 3.4 mmol/L (95% CI, 2.75-4.02) decreased to 1.74 mmol/L (95% CI, 1.32-2.16) after 1 week, followed by an increased over the next 2 weeks to 2.46 mmol/L (95% CI, 2.25-2.67) (figure 3d).

In the atypical lymphocyte trend group, 23 (21%) patients had “definite TBM and 89 (79%) “probable” TBM. Twenty-two (18%) of the 123 patients in the atypical neutrophil tend group had “definite TBM and the rest (82%) had “probable” TBM. Of the 152 patients with atypical

Stellenbosch University https://scholar.sun.ac.za

protein trends 24 (16%) had “definite” TBM and 128 (86%) “probable” TBM. 128. Similar to the atypical lymphocyte trend group, 22 (23%) of the patients with an atypical CSF glucose trend had “definite” TBM and the remaining 17 (79%), “probable” TBM.

Univariable analysis was performed to predict variables that were independently associated with these atypical CSF trends. Table 5 summarizes the findings.
A history of prolonged duration of illness >5 days (OR 2.30; 95% CI, 1.26-4.17; p=0.01), cranial nerve palsy (OR 1.77; 95% CI, 1.06-2.95; p=0.03) and CSF neutrophil predominance on admission (OR 2.90; 95% CI, 1.61-5.20; p<0.01) were significantly associated with an atypical CSF lymphocyte trend (CSF lymphocytes increasing between admission and follow-up CSF analysis up to 3 weeks post-admission). Similarly, a positive tuberculin skin test (OR 1.60; 95% CI 0.99-2.57; p=0.05) and a CSF lymphocyte count of >50% (OR 3.59; 95% CI 1.74-7.41; p<0.01) were significantly associated with an atypical CSF neutrophil trend (CSF neutrophils increasing between admission and follow-up CSF analysis up to 3 weeks post-admission). Only an admission CSF protein level >1g/L predicted an atypical trend of serially increasing CSF protein subsequent to admission (OR 3.30; 95% CI, 1.96-5.56; p<0.01). No variables were found to be associated with an abnormal CSF glucose trend.

In order to determine the value of serial CSF when scoring suspects using the uniform TBM research case definition, points for CSF features were allocated at admission and weeks 1 to 3. During the study period, in addition to the 318 children included with ‘definite’ and ‘probable’ TBM, there were 44 children identified with ‘possible’ TBM. In 6 of the children with ‘possible’ TBM at admission (14%), re-scoring following repeat CSF analysis improved their research case definition status to ‘probable’ TBM.

Longitudinal clustering was performed using the four CSF variables measured at four time points in our study population (n=318). There were only 91 patients with complete information in all 16 measurements (4 variables at 4 time points) k-medians partition cluster analysis was done to provide the classification in k groups based on the median profile across the 16 variables. To enhance the classification 9 more variables were added to the dataset. This was the difference between times 1 and 0, 2 and 1 and 3 and 2 for each of the four main CSF variables. This would

Stellenbosch University https://scholar.sun.ac.za

add in separating different possible profiles. The median partitioning was used since there were some outliers in the data i.e. CSF protein. Figure 4 shows the three median base clustering graphs.

The three-group median base clustering demonstrated distinct profiles with different trajectories over the first two time points. There is a low group (n=150), a start high group (n=88) and a middle to high group (n=80). The low group is about half of the sample (n=318) and the other two groups about 25% each. The changes between time points seemed to be an important feature between these three groups. This clustering analysis demonstrated that group 3 followed an "atypical" trend for TBM suspects. The CSF median for lymphocytes, neutrophils, and protein increased over the first week, in contrast to group 1 which followed the typical expected trend. The CSF glucose trends remained uniform amongst the three groups.

Stellenbosch University https://scholar.sun.ac.za

Chapter 6: Discussion

Repeat lumbar CSF does not improve the early diagnosis of childhood TBM, but it does affect the sensitivity of clinical confirmation in children diagnosed with TBM based on the uniform TBM research case definition.

In this study, serial lumbar CSF parameters analyzed in children with "probable" and "definite" tuberculous meningitis, showed a steady decline in lymphocyte and protein count, a more rapid decrease in neutrophil count, with a rapid increase in CSF glucose concentration, similar to adult studies.[25-27] CSF glucose concentration was the only parameter that normalized over the 3-week time period with concentrations returning to >2.2 mmol/L one week post-initiation of treatment. TBM is a chronic inflammatory response and CSF is expected to take weeks to normalize whilst rapid normalization is expected in non- tuberculous meningitis.[29]

Mycobacterial confirmation was found in 17% of initial CSF samples. This is in agreement with previous studies that found a frequency of 12-31% [7,13,14,24,35] and reflects normal clinical practice where most TBM cases will not have microbiologic confirmation of *.M.tb* due to the paucity of bacilli in the CSF. In contrast, one study had positive CSF microscopy or culture in 69% of patients after multiple samples were taken following initiation of treatment.[15] It has been recognized that sensitivity of acid-fast staining and *M.tb* culture is directly dependent on the number and volume of CSF samples.[15] Repeat CSF samples, unfortunately, did not include microscopy, culture or PCR, which could have influenced microbiological confirmation in our study.

The mean age of our study population was ±36 months, boys and girls being equally affected. This is comparable to several studies that indicate that TBM mainly effect children < 5years of age, a peak age between 24 and 48 months, with no gender preference. [6-8,13, 15, 22,24] A high incidence was seen amongst patients of mixed ancestry (79.4%), and no patients of European descent were part of the study. This population distribution is representative of our hospitals location and drainage area.

Stellenbosch University https://scholar.sun.ac.za

The HIV status was unfortunately only available in 35% of our patients with 4% being infected. This compares to literature that found 1-10% of patients being infected, [8, 22,24] but is in contrast to one study that found that 72% of microbiologically-confirmed TBM patients was HIV positive.[30] Our study time frame dates back to 1985, two years after Acquired Immunodeficiency Syndrome (AIDS) was diagnosed for the first time in South Africa, which probably contributes to the low number of patients tested for HIV. HIV-positive patients have a higher frequency of a non-inflammatory CSF profile that may influence the early diagnosis.[30] Co-infected patients also have a higher mortality rate and are more susceptible to MDR-TB, thus excluding them from our study. There was strong epidemiological evidence of history of contact with a household adult TB source case in 219 (69%) patients. This result is expected in a TB endemic environment like the Western Cape Province of South Africa. A background history with a known household TB source usually ranges between 30 -53 %.[7,13-15,24]

The most frequent symptoms and signs on admission included prolonged symptom duration >5 days, fever, meningeal irritation, altered level of consciousness and motor deficit, similar to previous studies. [7,13,15]A history of weight loss, defined as a decrease in weight, growth plateau, or slower weight gain to age- and sex-matched controls on the WHO weight for age charts, were found in less than half of our patients. This is not consistent with previous findings that 88-90% of patients had either weight loss or poor weight gain for weeks to months before presentation.[7,24] It is recognized that TBM is frequently accompanied by miliary tuberculosis, although the pathogenesis remains unclear.[11,14,24] Our study found a miliary CXR pattern in 12% of patients.

Based on refined British Medical Research Council (BMRC) criteria,[16,17] almost half of the patients presented with advanced disease (stage 3), similar to previous reports.[7,15] The delayed presentation, especially in our setting, can in part due to social reasons including lack of education, limited access to health-care facilities and also due to misinterpretation of the initial non-specific symptoms in young children by healthcare workers leading to delayed diagnosis. Tygerberg Hospital, as a tertiary referral institute, also tends to see patients with more advanced disease.

Stellenbosch University https://scholar.sun.ac.za

The study supports the typical characteristic lumbar CSF findings of a cell count of 10-500/ µL (87%) with lymphocytic predominance (82%), raised protein concentration of ≥ 1 g/L (72%) and CSF glucose ≤ 2.2 mmol/L, or CSF glucose/serum glucose ≤ 50% (70%).[7,13,19, 21-23]

Atypical fluctuations in CSF cell count, protein and glucose results are common during the early weeks of anti-tuberculous therapy.[13] Our study had a total of 230 (72%) patients with atypical CSF trends over the time period. A temporary deterioration of CSF findings does not necessarily indicate inappropriate therapy or a wrong diagnosis.

A history of prolonged illness >5days, cranial nerve palsy, and a CSF neutrophil predominance were significantly associated with an increase in CSF lymphocyte count over the 3 weeks. A positive tuberculin skin test and a CSF lymphocyte predominance on initial lumbar puncture were significantly associated with an atypical neutrophil trend increasing between admission and follow-up CSF analysis 3 weeks post-admission. An atypical CSF protein level >1g/L on admission predicted an atypical trend of serially increasing CSF protein over the time period.

Stellenbosch University https://scholar.sun.ac.za

Chapter 7:

Strengths

Our study included a large TBM sample size with extensive data that was collected prospectively. The data included CSF samples at different time points which makes it ideal for comparison and to assess trends over time. The data set has a high internal validity.

Limitations

As this was a retrospective evaluation of prospectively collected data, the biggest limiting factor was missing data. This ranged from relevant history of symptoms that was not taken or documented adequately, lumbar puncture that was not repeated or serum glucose not being measured for comparison.

The study was limited by the small number of patients with serum glucose measurements and HIV co-infection. Anti-retroviral therapy (ART) status of HIV infected patients was unknown. Important data that was not collected included a history of previous TB and BCG vaccination. External validity was poor and needs to be assessed. This may possibly lead to bias and not necessarily be representative of the more advantaged patients that have access to private healthcare.

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Chapter 8: Recommendations

1. In children with suspected TBM, serial CSF cultures may increase the diagnostic accuracy when the uniform research case definition for TBM is used.
2. In TBM suspects, the serum glucose should be measured with CSF analysis to assess the CSF to plasma glucose ratio
3. Clinicians should always maintain a high index of suspicion amongst all children presenting with non-specific symptoms, especially in TB endemic areas, and initiate TBM treatment early to prevent sequelae.
4. CSF analysis can be repeated after 4 weeks on TBM treatment to assess the response.

Stellenbosch University https://scholar.sun.ac.za

Chapter 9: Conclusion

Knowledge of the different CSF responses during the course of TBM therapy is important in clinical decision making. From our study, the evolution of lumbar CSF in patients with “probable” and “definite” TBM, provides a standardize trend/predicted model, for comparison of follow up CSF analysis. Repeat lumbar CSF in TBM suspects can demonstrate different trends over time. The typical trend is a gradual decline in CSF lymphocyte, neutrophil, and protein count whilst the CSF glucose rises steadily. This slow change in lymphocytic, protein and neutrophil count unfortunately limits their clinical use.

It is not abnormal for the CSF lymphocyte, neutrophil, and protein count to rise initially after treatment initiation, which can be seen as an atypical trend for TBM. We identified certain clinical features that are significantly associated with atypical CSF change over time. Serial lumbar CSF analysis improves the diagnosis of childhood tuberculous meningitis when the uniform TBM research case definition’s scoring system is applied. It however does not improve earlier diagnosis and outcome of TBM in children, and it could cause confusion if an atypical trend is found. CSF analysis on admission remains the most valuable diagnostic tool and repeat LP does not influence diagnostic accuracy significantly.

Stellenbosch University https://scholar.sun.ac.za

References

1. World Health Organization. Global tuberculosis Report 2015. Geneva, Switzerland: WHO, 2015.
2. Thwaites GE, van Toorn R, Schoeman J. Tuberculous meningitis: More questions, still too few answers. *Lancet Neurol* 2013;12: 999-1010.
3. Berenguer J, Moreno S, Lagunaetal F. Tuberculous meningitis in patients infected with the human immunodeficiency virus. *NEJM* 1992;326(10):668-72.
4. Farer LS, Lowell AM, Meador MP. Extrapulmonary tuberculosis in the United States. *Am J Epidemiol* 1979;109(2):205-17.
5. Madhi SA, Huebner RE, Doedens L, Aduc T, Wesley D, Cooper PA. HIV-1 co-infection in children hospitalized with tuberculosis in South Africa. *Int J Tuberc Lung Dis* 2000;4(5):448-54.
6. Thwaites G. Tuberculous meningitis. *Medicine.* 2013;41(12):683-5.
7. van Well GT, Paes BF, Terwee CB, et al. Twenty years of pediatric tuberculous meningitis: a retrospective cohort study in the western cape of South Africa. *Pediatrics* 2009;123: e1-8.
8. Nelson LJ, Schneider E, Wells CD, Moore M. Epidemiology of Childhood Tuberculosis in the United States, 1993-2001: The need for Continues Vigilance. *Pediatrics* 2004;114 (2):333-41.
9. Wolzak NK, Cooke ML, Orth H, van Toorn R. The changing profile of pediatric meningitis at referral centre in Cape Town, South Africa. *J Trop Pediatr*2012; 58:491-5.
10. Rich AR, McCordock HA. The pathogenesis of tuberculous meningitis. *Bull John Hopkins Hosp*1933; 52:5-37.
11. Donald PR, Schaaf HS, Schoeman JF. Tuberculous meningitis and miliary tuberculosis: The Rich focus revisited. *J Infect*2005; 50:193-5.
12. Thwaites G, Chau TTH, Mai NTH, Drobniewski F, McAdam K, Farrar J. Tuberculous meningitis. *J NeurolNeurosurg Psychiatry* 2000; 86:289-99.
13. Kumar R, Singh SN, Kohli N. A diagnostic rule for tuberculous meningitis. *Arch Dis Child*1999; 81:221-4.
14. Miftode EG, Dorneanu OS, Leca DA, et al. Tuberculous Meningitis in Children and Adults: A 10-Year Retrospective Comparative Analysis. *PLosone*2015;10(7): e0133477.

Stellenbosch University https://scholar.sun.ac.za

15. Farinha NJ, Razali KA, Holzel H, Morgan G, Novelli VM. Tuberculosis of the central nervous system in children: a 20-year survey. *J Infect*2000; 41:61-8.
16. British Medical Research Council. Streptomycin treatment of tuberculous meningitis. BMJ 1948; I: 582-97.
17. Van Toorn R, Springer P, Laubscher JA, Schoeman JF. Value of different staging systems for predicting neurological outcome in childhood tuberculous meningitis. *Int J Tuberc Lung Dis* 2012;16(5):628-32.
18. Donald PR, Schoeman JF. Central nervous system tuberculosis in children. In: Schaaf HS, Zumla AI (eds) Tuberculosis, a comprehensive clinical reference. Elsevier Publications, London, United Kingdom, 2009: Chapter 38; 413-423.
19. Youssef FG, AfifiSA, AzabAM, et al. Differentiation of tuberculous meningitis from acute bacterial meningitis using simple clinical and laboratory parameters. *Diagn Microbiol Infect Dis* 2006; 55: 275-8.
20. Rachow A, Clowes P, Saathoff E, et al. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: a prospective cohort study. Clin Infect Dis 2012; 54:1388-1396.
21. Marais S, Thwaites G, Marais JB et al. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infec Dis* 2010(10):803-12.
22. Solomons RS, Visser DH, Donald PR, Marais BJ, Schoeman JF, van Furth AM. The diagnostic value of cerebrospinal fluid chemistry results in childhood tuberculous meningitis. *Childs NervSyst*2015; 31:1335-40.
23. Thwaites GE, Hien TT. Tuberculous meningitis: many questions, too few answers. *Lancet Neurol*2005; 4:160-70.
24. Nabukeera-Barungi, N., Wilmshurst, J., Rudzani, M., & Nuttall, J. (2014). Presentation and outcome of tuberculous meningitis among children: experiences from a tertiary children's hospital. *African Health Sciences*, *14*(1), 143–9.
25. Kent SJ, Crowe SM, Yung A, Lucas CR, Mijch AM. Tuberculous meningitis: a 30-year review. *Clin Infect Dis*1993; 17:987-94.
26. Verdon R, Chevret S, Laissy JP, Wolff M. Tuberculous meningitis in adults: review of 48 cases. *Clin Infect Dis*1996; 22:982-8.
27. Thwaites GE, Simmons CP, Than Ha Quyen N, et al. Pathophysiology and prognosis in

Stellenbosch University https://scholar.sun.ac.za

Vietnamese adults with tuberculous meningitis. *J Infect Dis*2003; 188:1105-15.

28. Patel VB, Burger I, Connoly C. Temporal evolution of cerebrospinal fluid following initiation of treatment for tuberculous meningitis. *SAMJ* 2008;98;610-13.
29. Schoeman JF, Elshof JW, Laubscher JA, Janse van Rensburg A, Donald PR. The effect of adjuvant steroid treatment on serial cerebrospinal fluid changes in tuberculous meningitis. *Ann Trop Paediatr*2001; 21:299-30.
30. Cecchini, D., Ambrosioni, J., Brezzo, C., Corti, M., Rybko, A., Perez, M., … Ambroggi, M. (2009). Tuberculous meningitis in HIV-infected and non-infected patients: Comparison of cerebrospinal fluid findings. *International Journal of Tuberculosis and Lung Disease, 13*(2), 269–271.
31. Donald PR, Victor TC, Jordaan AM, Schoeman JF, van Helden PD. Polymerase chain reaction in the diagnosis of tuberculous meningitis. *ScandJInfect Dis*1993;25(5):613-7.
32. Iseman MD, A Clinician's Guide to Tuberculosis. Lippincott Williams & Wilkins, Baltimore, Maryland, USA, 1999.
33. Kennedy DH, Fallon RJ. Tuberculous meningitis. *JAMA*1979; 241:264-68.
34. Daniel TM. New approaches to the rapid diagnosis of tuberculous meningitis. *J Infect Dis*1987; 155:599-603.
35. World Health Organization. Xpert MTB/RIF: WHO Policy update and Implementation manual. WHO report 2013. Geneva, Switzerland.
36. Bahr NC, Marais S, Caws M, et al. GeneXpert MTB/Rif to Diagnose Tuberculous Meningitis: Perhaps the First Test but not the Last. *Clin Infect Dis.* 2016;62(9):1133-5.
37. Solomons RS, Visser DH, Friedrich SO, et al. Improved diagnosis of childhood tuberculous meningitis using more than one nucleic acid amplification test. *Int J Tuberc Lung Dis* 2015;19(1):74-80.

Stellenbosch University https://scholar.sun.ac.za

List of Tables

Table 1 Demographic data

Variable	
Age in months (mean (95% CI)	36.34(30.68-42.01)
Gender	
Male (n/N, %)[#]	165/317(52)
Female (n/N, %)[#]	152/317(48)
Ethnicity	
Black (n/N, %)[#]	65/316(21)
Mixed ancestry (n/N, %)[#]	251/316(79)
Positive HIV (n/N, %)[#]	4/111(4)
TB contact[*] (n/N, %)[#]	219/318(69)

CI = confidence interval; HIV = human immunodeficiency virus; TB = tuberculosis

[*] Defined as a history of recent close contact with a person with infectious TB within the past year

[#] The denominator varies according to available data

Stellenbosch University https://scholar.sun.ac.za

Table 2 Clinical features at presentation

Variable	Frequency (%)
Symptom duration >5 days	241/318(76)
Presenting symptoms	
History of fever[#]	187/296(63)
Weight loss[#]	127/296(43)
Vomiting[#]	155/296(53)
Convulsions[#]	131/296(44)
Cough >2 weeks	135/318(43)
Headache[#]	77/296(26)
FTT[+], night sweats, cough >2weeks	144/318(45)
Altered level of consciousness	303/318(95)
GCS on admission	
GCS 15[#]	13/317(4)
GCS 12-14[#]	128/317(40)
GCS 9-11[#]	72/317(23)
GCS <8[#]	104/317(33)
Meningeal irritation[#]	241/250(96)
Raised intracranial pressure[#]	78/316(25)
Brainstem dysfunction	123/318(39)
Cranial nerve palsies	81/318(26)
Focal neurological deficit[a]	201/318(63)
TBM Staging[*]	
Stage I	6/318(2)
Stage IIa	7/318(2)
Stage IIb	150/318(47)
Stage III	155/318(49)

TBM = tuberculous meningitis; FTT = failure to thrive; GCS = glascow coma scale

[*]Uniform research case definition from Marais et al.

[+] Defined as weight loss, growth plateau, or slower weight gain to age- and sex-matched controls on the WHO weight for age charts

[#] The denominator varies according to available data

[a] Includes hemiparesis, monoparesis and quadriplegia

Stellenbosch University https://scholar.sun.ac.za

Table 34 Results of other TB Investigations

Investigations	**Frequency** (%)
Tuberculin skin test reactive[#]	115/303(38)
CXR findings	
suggestive of PTB (excluding miliary TB)	155/318(49)
miliary TB	39/318(12)
Mtb culture positive gastric washings	62/318(19)
CT/MR results	
Cerebrovascular infarction	94/318(30)
Basal meningo-vascular enhancement	248/318(78)
Tuberculomas	38/318(12)
Hydrocephalus	293/318(92)

CXR = chest x-ray; PTB = pulmonary tuberculosis, TB = tuberculosis; Mtb = Mycobacterium Tuberculosis; CT = computed tomography; MRI = magnetic resonance imaging

[#]The denominator varies according to available data

Stellenbosch University https://scholar.sun.ac.za

Table 4: Cerebrospinal fluid findings on admission

Results	**Frequency(n/N) (%)**
Cells 10-500/µL	276/318(87)
Lymphocytes ≥ 50%	261/318(82)
Protein ≥ 1 g/L	229/316(72)
CSF:serum glucose ratio ≤ 50% or CSF glucose ≤ 2.2 mmol/L	222/303(73)
CSF microbiological confirmation Mtb*	53/318(17)

Mtb = Mycobacterium Tuberculosis

*Defined as a positive CSF culture, microscopy or PCR.

Stellenbosch University https://scholar.sun.ac.za

Table 5: Variables associated with an abnormal CSF trend

Characteristics	**OR (95% CI)**	**P value**
Atypical lymphocyte trend		
Prolonged duration of illness >5 days	2.30(1.26-4.17)	0.01
Cranial nerve palsy	1.77(1.06-2.95)	0.03
CSF neutrophil predominance on admission	2.90(1.61-5.20)	<0.01
Atypical neutrophil trend		
Positive tuberculin test	1.60(0.99-2.57)	0.05
CSF lymphocytes ≥ 50%	3.59(1.74-7.41)	<0.01
Atypical protein trend		
CSF protein ≥ 1 g/L on admission	3.30(1.96-5.56)	<0.01

Stellenbosch University https://scholar.sun.ac.za

List of Figures

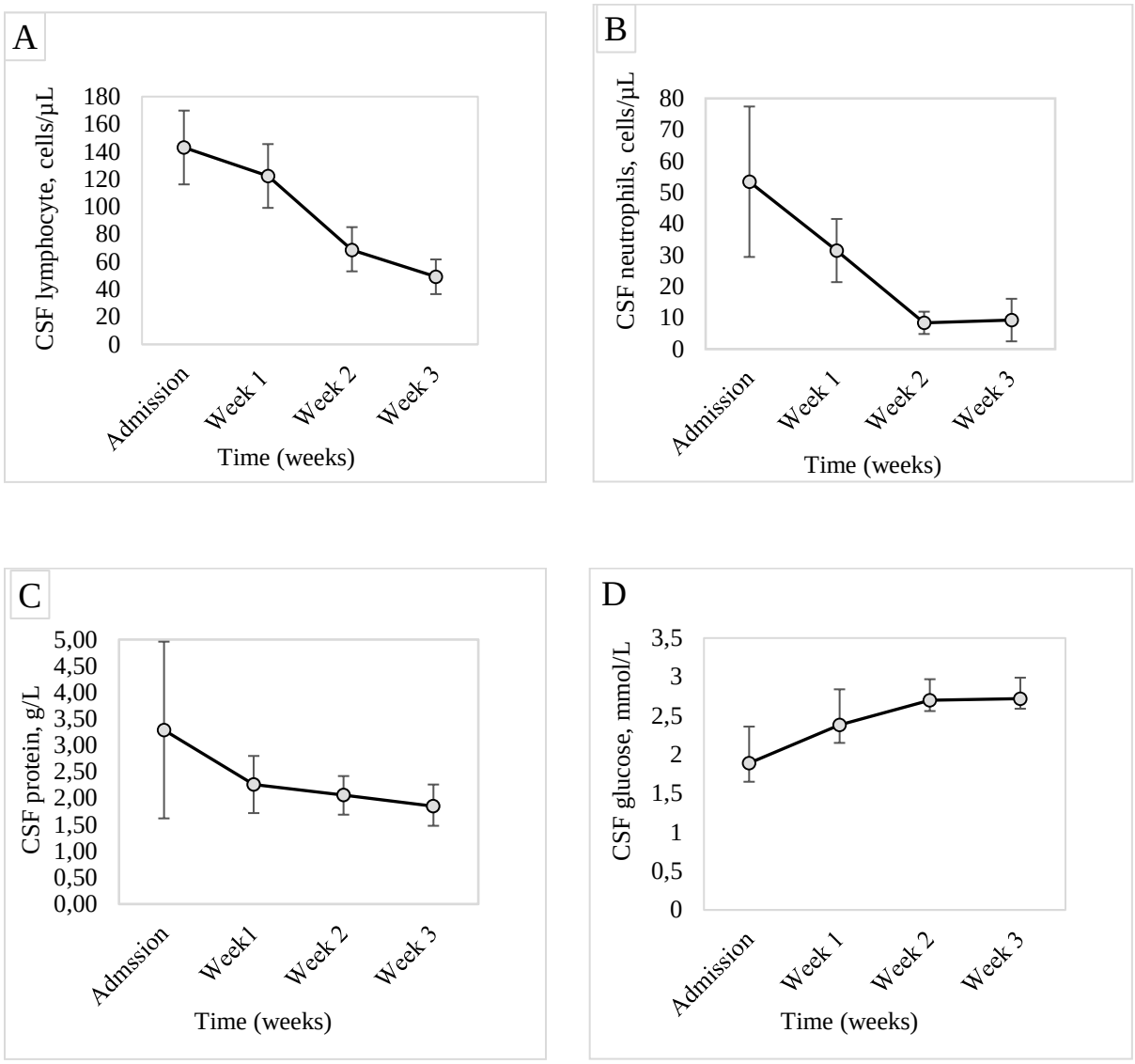


Figure 1. Mean values and 95% CI are shown of cerebrospinal fluid (CSF) lymphocyte count, neutrophil count, protein, and CSF glucose over the treatment period. A, Total CSF Lymphocyte over the treatment period. B, Total CSF neutrophil over the treatment period. C, Total CSF protein over the treatment period. D, Total CSF glucose over the treatment period.

Stellenbosch University https://scholar.sun.ac.za

Figure 2: Mean values and 95% CI are shown of all the typical cerebrospinal fluid (CSF) lymphocyte count, neutrophil count, protein, and CSF glucose over the treatment period.

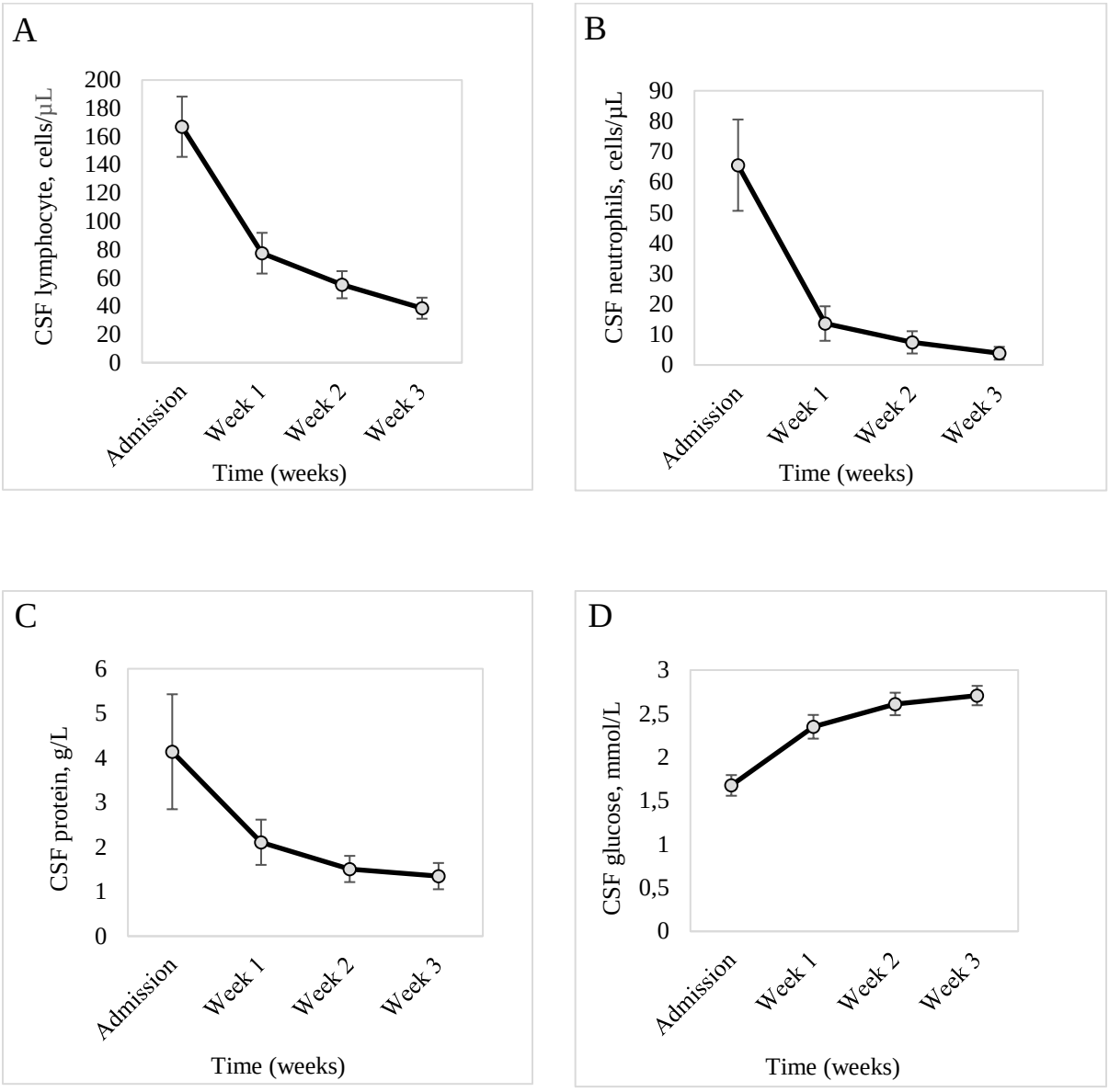


Figure 2. Mean values and 95% CI are shown of all the typical cerebrospinal fluid (CSF) lymphocyte count, neutrophil count, protein, and CSF glucose over the treatment period. A, Typical CSF Lymphocyte over the treatment period. B, Typical CSF neutrophil over the treatment period. C, Typical CSF protein over the treatment period. D, Typical CSF glucose over the treatment period.

Stellenbosch University https://scholar.sun.ac.za

Figure 3: Mean values and 95% CI are shown of all the atypical cerebrospinal fluid (CSF) lymphocyte count, neutrophil count, protein, and CSF glucose over the treatment period.

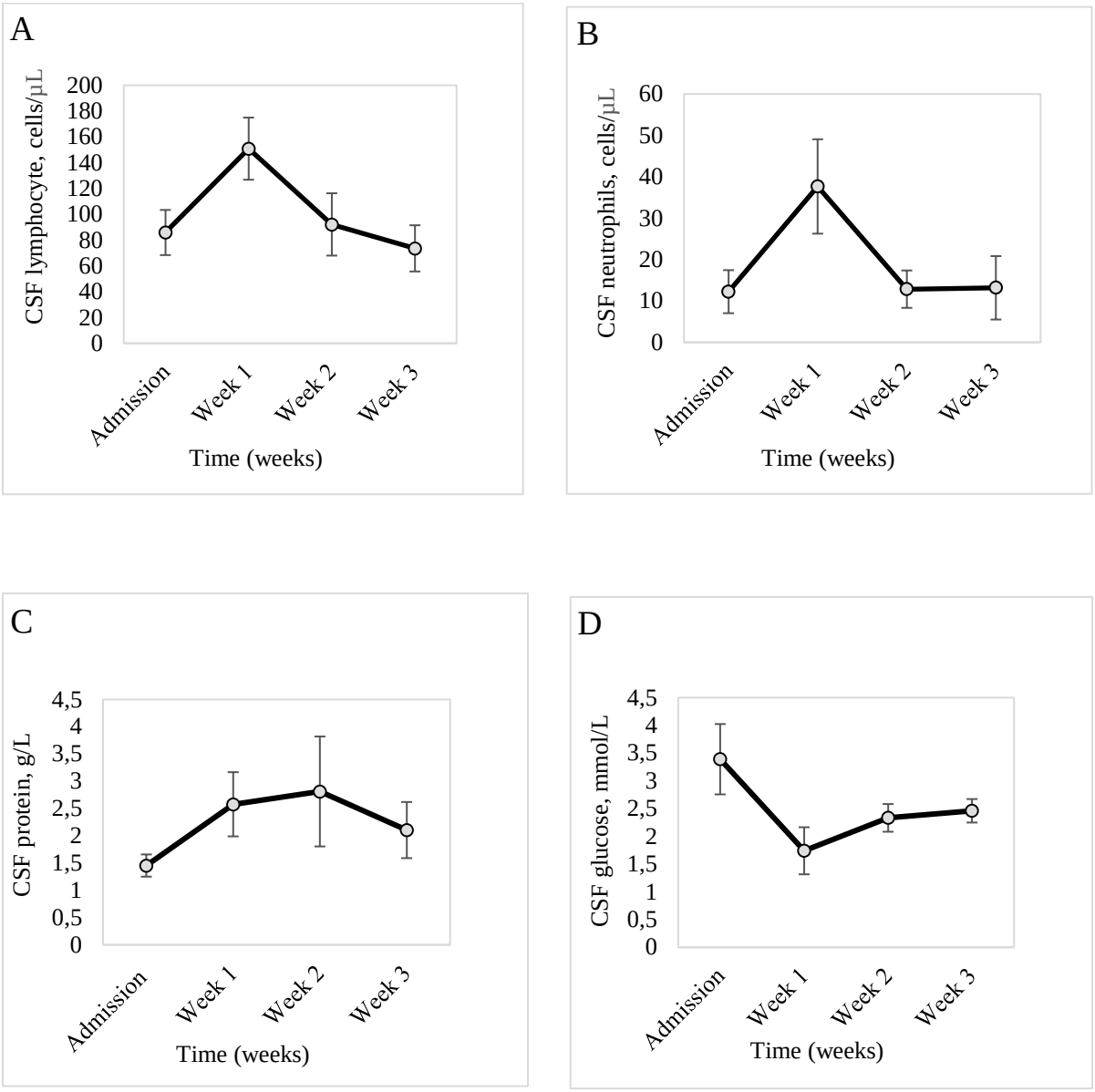


Figure 3. Mean values and 95% CI are shown of atypical cerebrospinal fluid (CSF) trends for lymphocyte count, neutrophil count, protein, and CSF glucose over the treatment period. A, Atypical CSF Lymphocyte trend over the treatment period. B, Atypical CSF neutrophil trend over the treatment period. C, Atypical CSF protein trend over the treatment period. D, Atypical CSF glucose trend over the treatment period.

Stellenbosch University https://scholar.sun.ac.za

Figure 4: Three group clustering median values of available cerebrospinal fluid (CSF) lymphocyte count, neutrophil count, protein, and CSF glucose over the treatment period.

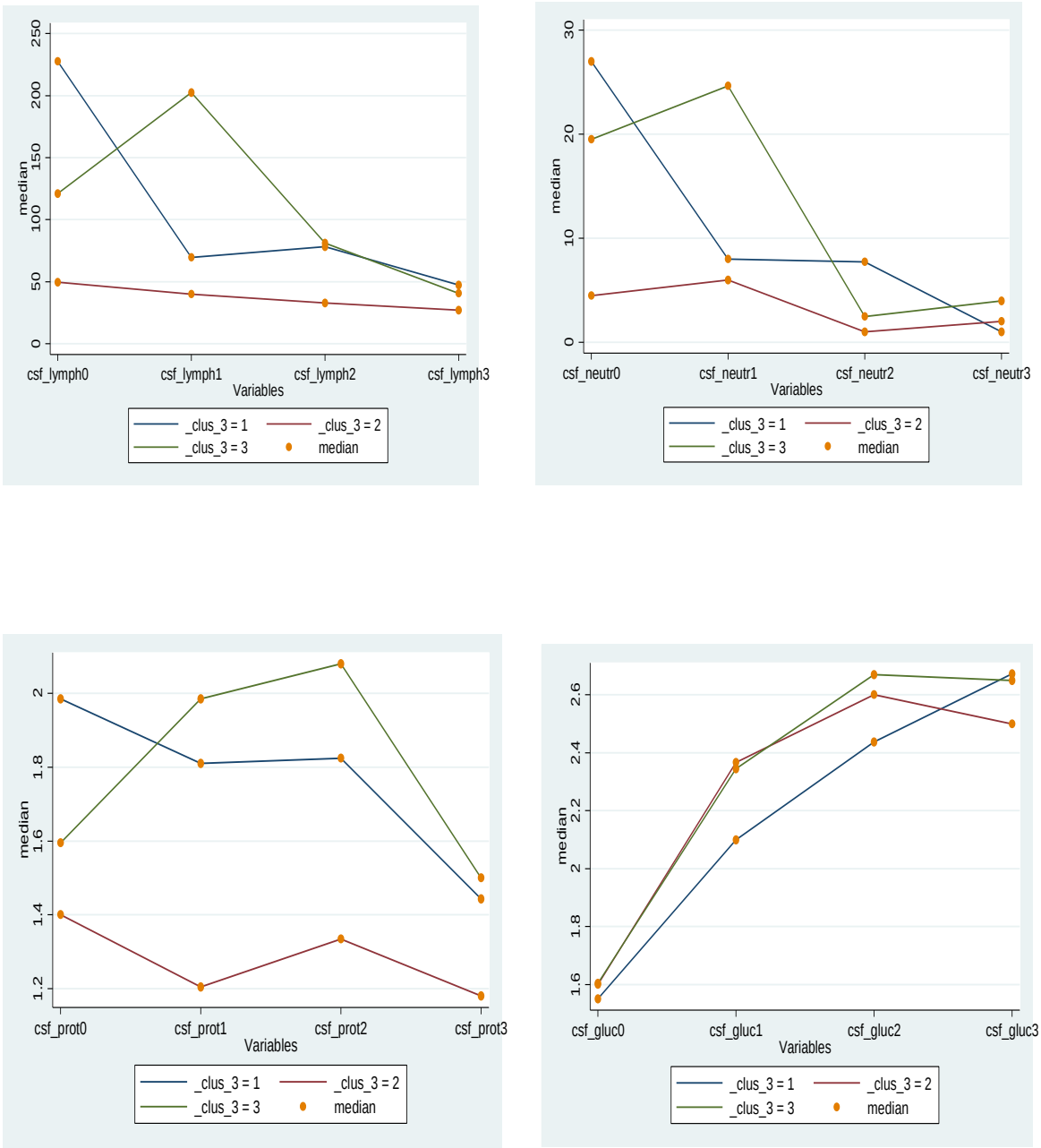


Figure 4. Three group clustering median values of available cerebrospinal fluid (CSF) lymphocyte count, neutrophil count, protein, and CSF glucose over the treatment period. A, CSF Lymphocyte over the treatment period. B, CSF neutrophil over the treatment period. C, CSF protein over the treatment period. D, CSF glucose over the treatment period.

Stellenbosch University https://scholar.sun.ac.za

List of Appendices

Appendix 1: Diagnosis of TBM: universal case definition[20]

	Diagnostic score
Clinical criteria (Maximum category score=6)	
Symptom duration of more than 5 days	4
Systemic symptoms suggestive of TB (1 or more of): weight loss/(poor weight gain in children), night sweats or persistent cough > 2 weeks	2
History of recent close contact with an individual with pulmonary TB or a positive TST/IGRA in a child <10 years	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
CSF criteria (Maximum category score=4)	
Clear appearance	1
Cells: 10–500 per μL	1
Lymphocytic predominance (>50%)	1
Protein concentration greater than 1 g/L	1
CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2.2mmol/L	1
Cerebral imaging criteria (Maximum category score=6)	
Hydrocephalus (CT and/or MRI)	1
Basal meningeal enhancement (CT and/or MRI)	2
Tuberculoma (CT and/or MRI)	2
Infarct (CT and/or MRI)	1
Pre-contrast basal hyperdensity (CT)	2
Evidence of tuberculosis elsewhere (Maximum category score=4)	
Chest radiograph suggestive of active TB (excludes miliary TB)	2
Chest radiograph suggestive of miliary TB	4
CT/ MRI/ US evidence for TB outside the CNS	2
AFB identified or *M. tuberculosis* cultured from another source i.e., sputum, lymph node, gastric washing, urine, blood culture	4
Exclusion of alternative diagnoses- An alternative diagnosis must be confirmed microbiologically, serologically or histopathologically	
Definite TBM =AFB seen on CSF microscopy, positive CSF *M. tuberculosis* culture, or positive CSF*M. tuberculosis* commercial NAAT in the setting of symptoms/signs suggestive of meningitis; or AFB seen in the context of histological changes consistent with TB brain or spinal cord together with suggestive symptoms/signs and CSF changes, or visible meningitis (on autopsy).	
Probable TBM = total score of ≥12 when neuroimaging available = total score of ≥10 when neuroimaging unavailable	
Possible TBM = total score of 6-11 when neuroimaging available = total score of 6-9 when neuroimaging unavailable	

Stellenbosch University https://scholar.sun.ac.za

Appendix 2: Data collection sheet

	Admission	Week 1	Week 2	Week 3
Basic information				
Inclusion number				
Age in months				
Gender				
Definite or probable TBM				
TBM stage				
Duration of symptoms (days)				
HIV status				
Neuroimaging				
Hydrocephalus y/n				
Communicating/Non-communicating				
Basal enhancement y/n				
Infarction y/n				
CSF analysis				
CSF volume taken (ml)				
CSF macroscopic appearance				
CSF Polymorphs cell/mm^3				
CSF Lymphocytes cell/mm^3				
CSF protein g/L				
CSF glucose mmol/L				
Other investigations				
Extra neural B culture pos/neg				
Miliary TB CXR y/n				

Stellenbosch University https://scholar.sun.ac.za

Appendix 3: Ethical approval: S16/08/159

Approval Notice

Response to Modifications- (New Application)

16-Nov-2016
Grobbelaar, Madelein M

Ethics Reference #: S16/08/159

Title: **The value of repeat lumbar cerebrospinal fluid analysis in the diagnosis of childhood TBM**

Dear Dr Madelein Grobbelaar,

The **Response to Modifications -** ***(New Application)*** received on **28-Sep-2016**, was reviewed by members of **Health Research Ethics Committee 2** via Expedited review procedures on **16-Nov-2016** and was approved.
Please note the following information about your approved research

protocol: Protocol Approval Period: **16-Nov-2016 -15-Nov-2017**

Please remember to use your **protocol number** (**S16/08/159**) on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review:
Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.
Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States
Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western

Stellenbosch University https://scholar.sun.ac.za

Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel:
+27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.
For standard HREC forms and documents please visit:

www.sun.ac.za/rds If you have any questions or need further

assistance, please contact the HREC office at .

Included Documents:

Declaration R Solomons.pdf
20160930 MOD Protocol
Synopsis Declaration R van
Toorn.pdf
20160930 MOD HREC mods letter
Checklist.doc
Declaration M Grobbelaar.pdf
CV M Grobbelaar .doc
20160930 MOD HPCSA R Solomon
Protocol.docx
CV R Solomons.doc
Application form.pdf
20160930 MOD HPCSA M Grobbelaar
20160930 MOD Checklist
20160930 MOD Cover letter
Protocol Synopsis.docx
20160930 MOD HPCSA R van Toorn
CV Ronald van Toorn.doc
20160930 MOD Protocol

Sincerely,

Francis Masiye
HREC Coordinator
Health Research Ethics Committee 2

Stellenbosch University https://scholar.sun.ac.za